Types of Antigen–Antibody Reactions

Serological tests are widely used for detection of either serum antibodies or antigens for diagnosis of a wide variety of infectious diseases.

These serological tests are also used for diagnosis of autoimmune diseases and in typing of blood and tissues before transplantation. The following are the examples of antigen–antibody reactions:

a- precipitation

b- agglutination

c- complement-dependent serological tests

d- neutralization test

e- opsonization

f - immunofluorescence

g- enzyme immunoassay

h-radioimmunoassay

Precipitation: involves combination of soluble antibody with soluble antigen to produce insoluble complexes.

Precipitation Curve: Precipitation reactions are also dependent on the amount of antigen and antibody present in the test system.

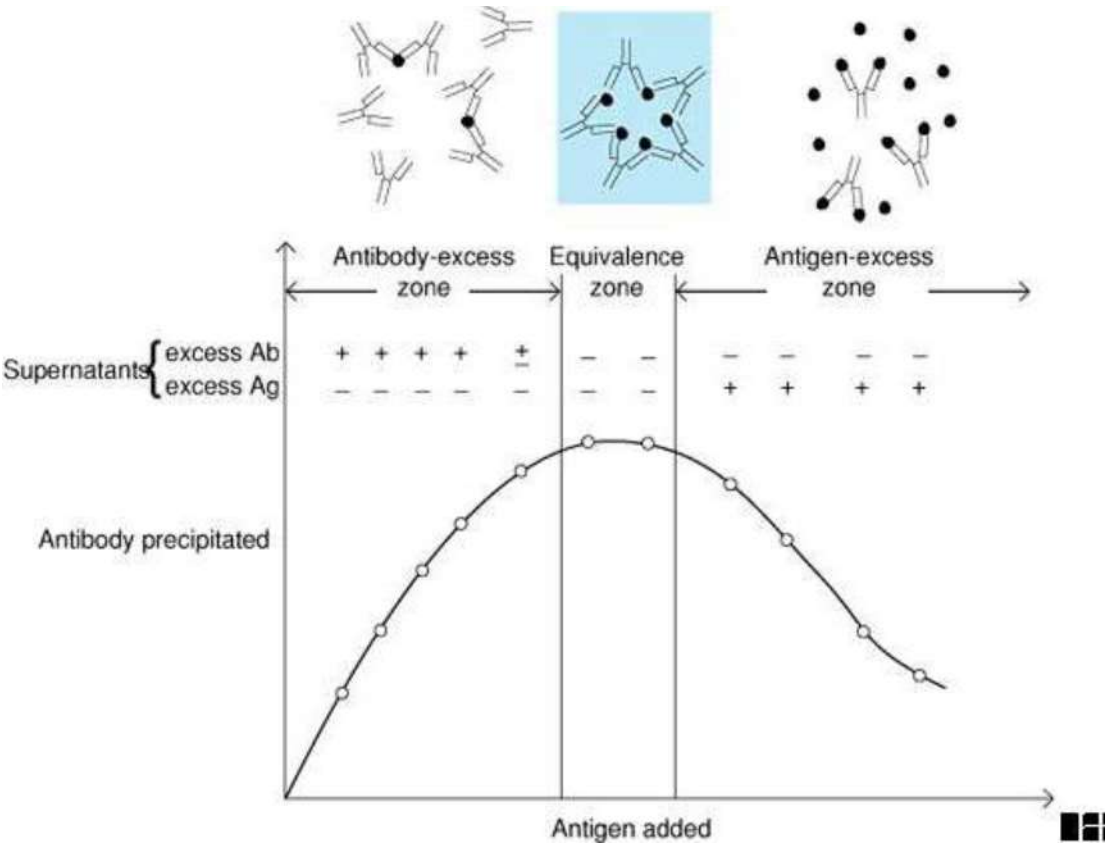
1 - **Prozone phenomenon**: occurs when excess antibody is present. So much antibody is present that all antigen sites are coated and lattice formation cannot occur.

2- **Postzone phenomenon**: occurs when excess antigen is present.

a. There is so much antigen present that the antibody molecules bind in such a way that lattice formation cannot occur.

b. The antibody may bind to antigen sites on two separate molecules, sensitization may occur, but the binding is not on enough adjacent molecules to form lattice.

3 - **Zone of equivalence** : is when antigen and antibody are present in optimal proportions, sufficient antibodies can bind to antigens on adjacent molecules resulting in lattice formation.



Applications of precipitation:

١- identification of bacteria. ٢- identification of bacterial components in infective tissues. ٣- detection of unknown antibody.

Immunodiffusion Techniques

Antigen and antibody reactions occur in a gel, migrating towards each other and forming a detectable precipitate in the gel.

The rate of diffusion is affected by:

a. Size of the particles b. Temperature
c. Gel viscosity and hydration d. Interaction of the reactants with the gel
Classified into 4 types:

a. Single diffusion (one reactant moving) single dimension (up or down).

b. Single diffusion, double dimension (moving our radially from a well).

c. Double diffusion (both reactants moving) single dimension.

d. Double diffusion, double dimension Oudin - (single diffusion/single dimension)

١- **Oudin** first one to employ gels.

2- Antibody is added to agarose gel and placed in a tube, antigen is layered on top of the gel and will diffuse down into the gel.

3- If the antibody present reacts with the added antigen a precipitin band will form in the gel.

Uses: It is used for detection of number of Ags present in a mixture.

Radial Immunodiffusion-RID (single diffusion/double dimension)

1- Antibody is added to the gel and poured into a plate, wells are cut into the plate.

2- Antigen is added to the well and will diffuse out radially from the well.

3- If the antibody present is specific for the antigen added a ring of precipitate will form, the size of the ring is directly proportional to the concentration of the antigen.

4- Standards are run at the same time and a standard curve is created.

Uses: 1-for the quantitation of soluble Ags in body fluid

2- for the quantitation of immunoglobulin ,plasma proteins and complement components.

Ouchterlony Gel Diffusion (double diffusion/double dimension)

1- Ouchterlony Immunodiffusion is a method used for comparison of antigens.
2- Holes are cut in the agar, one central hole surrounded by other wells.
3- Antibody is added to the central well, antigens are added to the outer wells, the position of the bands formed between the antigens allows for comparison of the antigens to each other.

Uses: 1-Detection of an Ags 2- Comparison of two Ab-Ag system.

3- Diagnosis of bacterial disease. 4- Diagnosis of viral disease.

5- Diagnosis of fungal disease. 6- Diagnosis of parasitic disease.

Agglutination: is an antigen–antibody reaction in which a particulate antigen combines with its antibody in the presence of electrolytes at a specified temperature and pH resulting in formation of visible clumping

of particles. Agglutination occurs optimally when antigens and antibodies react in equivalent proportions.

Types of agglutination reactions

Direct agglutination

Direct agglutination reactions can broadly be of the following types: (a) slide agglutination, (b) tube agglutination, (c) heterophile agglutination, and (d) antiglobulin (Coombs') test.

Passive agglutination

Passive agglutination reaction, depending on the carrier particles used, can be of the following types: (i) latex agglutination test, (ii) hemagglutination test,and (iii) coagglutination test.

Complement-Dependent Serological Tests:

The complement-dependent serological tests may be of the following

types:

1- Complement fixation test ٢- Immune adherence test

٣-Immobilization test ٤-Cytolytic or cytocidal reactions

Neutralization Tests:

Neutralization is an antigen–antibody reaction in which the biological effects of viruses and toxins are neutralized by homologous antibodies known as neutralizing antibodies.

These tests are broadly of two types: (a) virus neutralization tests and

(b) toxin neutralization tests.

Opsonization: is a process by which a particulate antigen becomes more susceptible to phagocytosis when it combines with opsonin.

Immunofluorescence

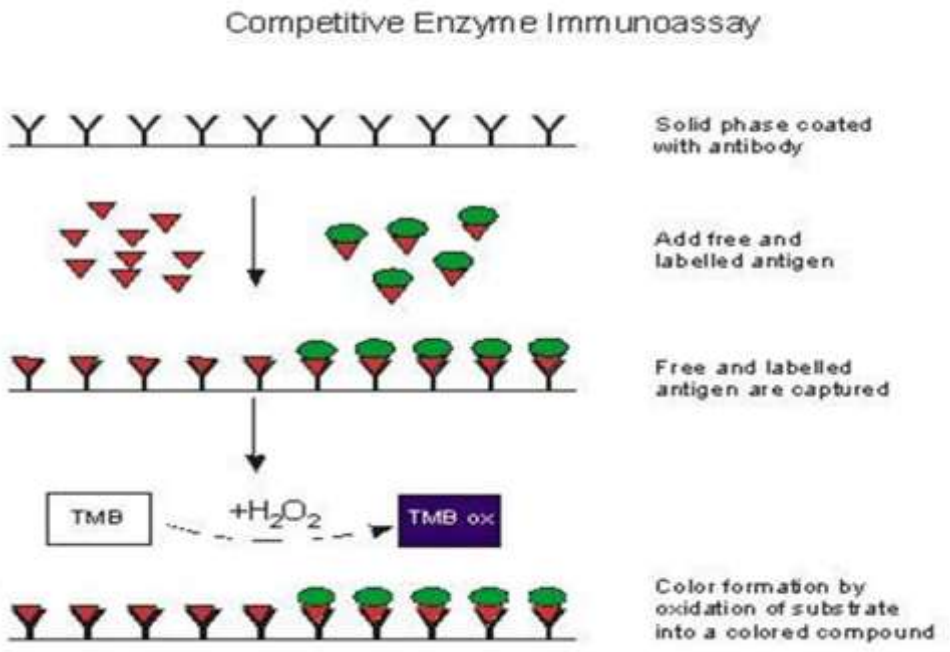
The property of certain dyes absorbing light rays at one particular wavelength (ultraviolet light) and emitting them at a different wavelength (visible light) is known as fluorescence. Immunofluorescence tests have wide applications in research and diagnostics. These tests are broadly of two types:

1- Direct immunofluorescence test ٢- Indirect immunofluorescence test

ELISA technique:
Is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample
The technique is divided into:-
1- Competitive ELISA
2- Sandwich ELISA (also called direct ELISA)
3- Indirect ELISA

Competitive ELISA

The labelled antigen competes for primary antibody binding sites with the sample antigen (unlabeled). The more antigen in the sample, the less labelled antigen is retained in the well and the weaker the signal.



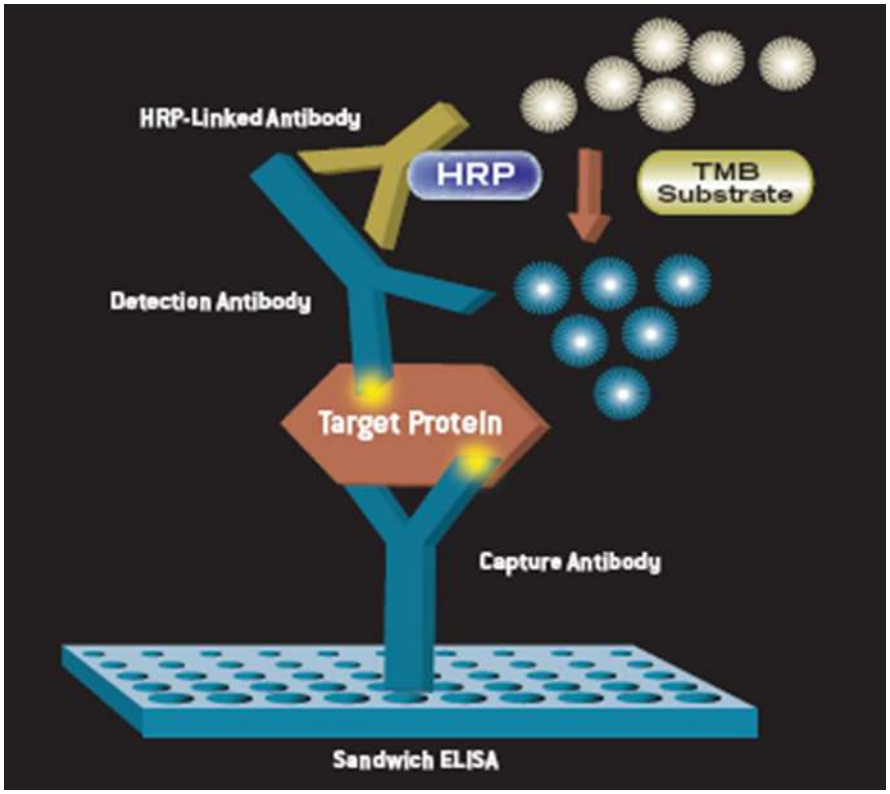
Sandwich ELISA

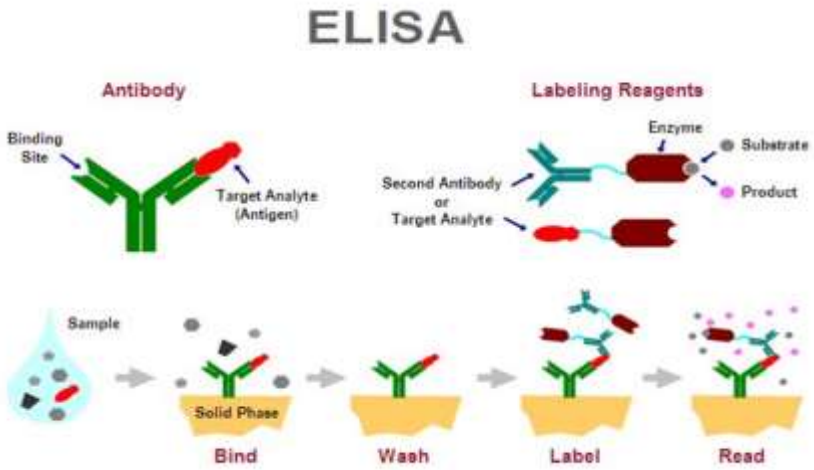
The ELISA plate is coated with Antibody to detect specific antigen.

1-Prepare a surface to which a known quantity of capture antibody is bound.

2- Block any non specific binding sites on the surface.
3- Apply the antigen-containing sample to the plate.
4- Wash the plate, so that unbound antigen is removed.
5- Apply enzyme linked primary antibodies as detection antibodies which also bind specifically to the antigen.
6- Wash the plate, so that the unbound antibody-enzyme conjugates are removed.

7- Apply a chemical which is converted by the enzyme into a coloured product.
8- Measure the absorbency of the plate wells to determine the presence and quantity of antigen.





Indirect ELISA

The protein antigen to be tested for is added to each well of ELISA plate, where it is given time to adhere to the plastic through charge interactions

A solution of non-reacting protein is added to block any plastic surface in the well that remains uncoated by the protein antigen

Then the serum is added, which contains a mixture of the serum antibodies, of unknown concentration, some of which may bind specifically to the test antigen that is coating the well.

Afterwards, a secondary antibody is added, which will bind to the antibody bound to the test antigen in the well. This secondary antibody often has an enzyme attached to it.

A substrate for this enzyme is then added. Often, this substrate changes colour upon reaction with the enzyme. The colour change shows that secondary antibody has bound to primary antibody, which strongly implies that the donor has had an immune reaction to the test antigen.

The higher the concentration of the primary antibody that was present in the serum, the stronger the colour change. Often a spectrometer is used to give quantitative values for colour strength.